IN THE SPECIFICATION

Page 1, line 2, after the title, insert the following new section:

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a continuation of U.S. application 09,402,277, filed September 30, 1999, now abandoned, which is a §371 of PCT/GB98/00961, filed April 1, 1998, the entire contents of each being incorporated herein.

Replace the paragraph beginning on page 18, line 8, with the following rewritten paragraph:

~~FIGURE 1 illustrates~~ Figure 1A and Figure 1B illustrate a method for the simultaneous amplification and immobilisation of nucleic acid molecules using a single type of primer.

Replace the paragraph beginning on page 18, line 20, with the following rewritten paragraphs:

Figure 5 is an example of DNA colonies formed by amplification of a specific template with single primers grafted onto a surface.

~~FIGURE 5 illustrates~~Figures 6A and Figure 6B illustrate a method for the simultaneous amplification and immobilisation of nucleic acid molecules using two types of primer.

Replace the paragraph beginning on page 18, line 23, with the following rewritten paragraph:

~~FIGURE 6~~Figure 7 shows actual DNA colonies produced via the present invention.

Replace the paragraph beginning on page 18, line 25, with the following rewritten paragraphs:

Figure 8 shows actual DNA colonies produced via the present invention.

~~FIGURE 7 illustrates~~Figure 9A and Figure 9B illustrate a method of the simultaneous amplification and immobilisation of nucleic acid molecules when a target molecule is used as a template having internal sequences that anneal with primers.

Replace the paragraph beginning on page 19, line 1, with the following rewritten paragraph:

~~Figure 8 illustrates~~Figure 10A and Figure 10B illustrate a method to synthesise additional copies of the original nucleic acid strands using nucleic acid strands present in colonies. The newly synthesised strands are shown in solution but can be provided in immobilised form if desired.

Replace the paragraph beginning on page 19, line 5, with the following rewritten paragraph:

~~Figure 9 shows~~Figure 11A and Figure 11B show the PCR amplification of DNA from DNA found in the pre-formed DNA colonies.

Replace the paragraph beginning on page 19, line 7, with the following rewritten paragraph:

~~FIGURE 10 illustrates~~Figure 12A, Figure 12B, and Figure 12C illustrate how secondary primers can be generated from DNA colonies.

Replace the paragraph beginning on page 19, line 9, with the following rewritten paragraph:

~~FIGURE 11 illustrates~~Figure 13A and Figure 13B illustrate how secondary DNA colonies can be generated from secondary primers.

Replace the paragraph beginning on page 19, line 11, with the following rewritten paragraph:

~~FIGURE 12 illustrates~~Figure 14A and Figure 14B illustrate how primers with different sequences can be generated from a surface functionalised with existing primers.

Replace the paragraph beginning on page 19, line 14, with the following rewritten paragraph:

~~FIGURE 13~~Figure 15 depicts methods of preparing DNA fragments suitable for generating DNA colonies.

Replace the paragraph beginning on page 19, line 16, with the following rewritten paragraph:

~~FIGURE 14~~Figure 16 illustrates a method for synthesising cRNA using the DNA colony as a substrate for RNA polymerase.

Replace the paragraph beginning on page 19, line 18, with the following rewritten paragraph:

~~FIGURE 15~~Figure 17 illustrates a preferable method to determine the DNA sequence of DNA present in individual colonies.

Replace the paragraph beginning on page 19, line 20, with the following rewritten paragraph:

~~FIGURE 16~~Figure 18 illustrates a method of determining the sequence of a DNA colony, de novo.

Replace the paragraph beginning on page 19, line 22, with the following rewritten paragraph:

~~FIGURE 17~~Figure 19 illustrates the utility of secondary DNA colonies in the assay of mRNA expression levels.

Replace the paragraph beginning on page 19, line 24, with the following rewritten paragraph:

~~FIGURES 18 and 19 illustrates~~Figure 20 and Figure 21 illustrate the use of the secondary DNA colonies in the isolation and identification of novel and rare expressed genes.

Replace the paragraph beginning on page 23, line 8, with the following rewritten paragraph:

Oligonucleotides, phosphorylated at their 5'-termini (Microsynth GmbH, Switzerland), were grafted onto Nucleolink plastic microtitre wells (Nunc, Roskilde, Denmark). The sequence of the oligonucleotide p57 corresponds to the sequence 51-TTTTTTCACCAACCCAAACCAACCCAAACC (SEQ ID NO:1) and p58 corresponds to the sequence 51-TTTTTTAGAAGGAGAAGGAAAGGGAAAGGG (SEQ ID NO:2). Microtitre wells with p57 or p58 were prepared as follows. In each Nucleolink well, 30 μl of a 160nM solution of the oligonucleotide in 10 mM 1-methyl-imidazole (pH 7.0) (Sigma Chemicals, St. Louis, MO) was added. To each well, 10 μl of 40 mM 1-ethyl-3-(3dimethylaminopropyl)-carbodiimide (pH 7.0) (Sigma Chemicals) in 10 mM 1-methyl-imidazole, was added to the solution of oligonucleotides. The wells were then sealed and incubated at 50°C overnight. After the incubation, wells were rinsed twice with 200 μl of RS (0.4N NaOH, 0.25% Tween 20 (Fluka Chemicals, Switzerland)), incubated 15 minutes with 200 pl RS, washed twice with 200 μl RS and twice with 200 μl TNT (100mM TrisHCl pH7.5, 150mM NaCl, 0.1% Tween 20). Tubes were dried at 50°C and were stored in a sealed plastic bag at 4°C.

Replace the paragraph beginning on page 23, line 25, with the following rewritten paragraph:

Colony generation was initiated in each well with 15 μl of priming mix; 1 nanogram template DNA (where the template

DNA began with the sequence 51-AGAAGGAGAAGGAAAGGGAAAGGG (SEQ ID NO:3) and terminated at the 31-end with the sequence CCCTTTCCCTTTCCTTCTCCTTCT-3' (SEQ ID NO:4)), the four dNTPs (0.2 mM), 0.1% BSA (bovine serum albumin, Boehringer-Mannheim, Germany), 0.1% Tween 20, 8% DMSO (dimethylsulfoxide, Fluka Chemicals, Switzerland), 1X Amplitaq PCR buffer and 0.025 units/μl of AmpliTaq DNA polymerase (Perkin Elmer, Foster City, CA). The priming reaction was a single round of PCR under the following conditions; 94°C for 4 minutes, 60°C for 30 seconds and 72°C for 45 seconds in a thermocycler (PTC 200, MJ Research, Watertown, MA). Then 100 μl TE buffer (10mM trisHC1, pH 7.5, 1mM EDTA) was used in three successive one minute long washes at 94°C. The DNA colonies were then formed by adding to each well, 20 μl of polymerisation mix, which was identical to the priming mix but lacking the template DNA. The wells were then placed in the PTC 200 thermocycler and colony growing was performed by incubating the sealed wells 4 minutes at 94°C and cycling for 50 repetitions the following conditions: 94°C for 45 seconds, 65°C for 2 minutes, 72°C for 45 seconds. After completion of this program, the wells were kept at 8°C until further use.

Replace the paragraph beginning on page 24, line 11, with the following rewritten paragraph:

A 640 base pair fragment corresponding to the central sequence of the template (but not including the 5'-AGAAGGAGAAGGAAAGGGAAAGGG (SEQ ID NO:3) sequence) was amplified by PCR. The isolated fragment was ~~labelled~~ labeled with biotin-N^4-dCTP (NEN Life Sciences, Boston, MA) and a trace of [α-^{32}P]dCTP (Amersham, UK) using the Prime-it II ~~labelling~~labeling kit (Stratagene, San Diego, CA) to generate a biotinylated probe.

Replace the paragraph beginning on page 25, line 8, with the following rewritten paragraph:

~~Figure 4 shows~~Figures 4 and 5 show the hybridisation results for colony generation on tubes functionalised with either; ~~(a)~~Fig. 4 oligonucleotide p57 or ~~(b)~~Fig. 5 oligonucleotide p58. The control reaction shows very few fluorescent spots, since the sequence of the flanking regions on the template do not correspond to the primer sequences grafted onto the well. In contrast, ~~figure 4b~~Figure 5 shows the number of fluorescent spots detected when the primers grafted to the wells match the flanking sequences on the initiating DNA template. Calculating the number of fluorescent spots detected and taking into consideration the magnification, we can estimate that there are between 3 and 5 x 10^7 colonies /cm^2. The photos are generated by the program,

Winview 1.6.2 (Princeton Instruments, Trenton, NJ) with backgrounds and intensities normalised to the same values.

Replace the paragraph beginning on page 26, line 4, with the following rewritten paragraph:

Referring now to ~~Figure 5~~Figures 6A and 6B, another embodiment of the present invention is illustrated. Here two different immobilised primers are used to provide primer extension.

Replace the paragraph beginning on page 26, line 7, with the following rewritten paragraph:

In this embodiment the target molecule shown is provided with a nucleotide sequence at its 3' end (AAT-3') that is complementary to the sequence of a first primer, (5'-ATT, I), which is grafted on the surface, so-that annealing with that primer can occur. The sequence (5'-GGT) at the 5' end of the target molecule, III, corresponds to the sequence (5'-GGT) of a second primer, II, which is also grafted to the surface, so that the sequence which is complementary to the sequence at the 5' end can anneal with that said second primer. Generally said complementary sequence (5'-ACC) is chosen so that it will not anneal with the first primer (5'-ATT). Unlike the situation described in section A, once the 3' end of a newly synthesised strand anneals to a primer on the surface, it will have to find a primer whose sequence is

different from the sequence it carries at its 5' end (see the difference between ~~Figures 1f and 5f~~Figure 1A, (f) and Figure 6A, (f)).

Replace the paragraph beginning on page 26, line 21, with the following rewritten paragraph:

The embodiment shown in ~~Figure 5 has~~Figures 6A and 6B have an advantage over the embodiment illustrated in Figure 1 since the possibility of one end of a single stranded target molecule annealing with another end of the same molecule in solution can be avoided and therefore amplification can proceed further. The possibility of annealing occurring between both ends of an immobilised complement to a target molecule can also be avoided.

Replace the paragraph beginning on page 27, line 2, with the following rewritten paragraph:

A mix of two oligonucleotides which are phosphorylated at the 5'-end (Microsynth GmbH, Balgach, Switzerland) have been grafted on 96 well Nucleolink plates (Nunc, Denmark) as recommended by the manufacturer. The resulting plates has been stored dry at 4°C. The sequence of the primer, P1, was 5'-GCGCGTAATACGACTCACTA (SEQ ID NO:5), the sequence of the other primer, P2, was 5'-CGCAATTAACCCTCACTAAA (SEQ ID NO:6). These plates are specially formulated by Nunc,

allowing the covalent grafting of 51phosphorylated DNA fragments through a standard procedure.

Replace the paragraph beginning on page 28, line 4, with the following rewritten paragraph:

In ~~figure 6A~~Figure 7, the control well (without added DNA template, panel a) shows only rare objects which can be observed on a blank surface (these objects were useful at this stage for reporting that the focus was correct). These objects have an irregular shape, are 20 to 100 micro-meters in size and have a thickness much larger than the field depth of the observation. In a well where DNA polymerase was present (Figure 7~~figure 6A~~, panel ii), in addition to the objects of irregular shape observed in the control well, a great number of fluorescent spots can be observed. They present a circular shape, they are 1 to 5 micro meters in size and do not span the field of view. The number of spots depends on the concentration of the template used for initiating colony formation. From the observed size of the colonies, one can estimate that more than 10,000 distinct colonies can be arrayed within 1 mm^2 of support.

Replace the paragraph beginning on page 28, line 19, with the following rewritten paragraph:

Oligonucleotides (Microsynth GmbH, Switzerland) were grafted onto Nucleolink wells (Nunc, Denmark).

Oligonucleotide P1 corresponds to the sequence 5'-TTTTTTCTCACTATAGGGCGAATTGG (SEQ ID NO:7) and oligonucleotide P2 corresponds to 5'-TTTTTTCTCACTAAAGGGAACAAAAGCTGG (SEQ ID NO:8). In each Nucleolink well, a 45 μl of 10 mM 1-methyl-imidazole (pH 7.0) (Sigma Chemicals, St. Louis, MO) solution containing 360 fmol of P1 and 360 fmol of P2 was added. To each well, 15 μl of 40 mM 1-ethyl-3-(3-dinethylaminopropyl)-carbodiimide (pH 7.0) (Sigma Chemicals) in 10 mm 1-methyl-imidazole, was added to the solution of oligonucleotides. The wells were then sealed and incubated at 50°C for 16 hours. After the incubation, wells have been rinsed twice with 200 μl of RS (0.4N NaOH, 0.25% Tween 20), incubated 15 minutes with 200 μl RS, washed twice with 200 μl RS, and twice with 200μl TNT (100mM Tris/HCl pH7.5, 150mM NaCl, 0.1% Tween 20), before they are put to dry at 50°C in an oven. The dried tubes were stored in a sealed plastic bag at 4°C.

Replace the paragraph beginning on page 30, line 20, with the following rewritten paragraph:

The ~~figure 6B~~ Figure 8 shows the results for 3 different ratios of the S1/S2 templates used in the initiating reaction: i) the S1/S2 is 1/0, many spots can be observed, ii) the S1/S2 is 1/10, and the number of spots is approximately 1/10 of the number of spots which can be observed in the i)

image, as expected, and iii) the S1/S2 is 0/1, and only a few rare spots can be seen.

Replace the paragraph beginning on page 31, line 4, with the following rewritten paragraph:

~~Figure 7 is~~Figures 9A and 9B are provided to show that the sequences shown at the 5' and 3' ends of the target molecule illustrated in ~~Figures 1 and 5~~Figures 1A and 1B and Figures 6A and 6B need not be located at the ends of a target molecule.

Replace the paragraph beginning on page 31, line 7, with the following rewritten paragraph:

A target nucleic acid molecule (II) may have a sequence at each (or either) end that is neither involved in annealing with a primer nor in acting as a template to provide a complementary sequence that anneals with a primer (sequence 5'-AAA and sequence 5'-CCC). One of the internal sequences (5'-AAT) is used as a template to synthesise a complementary sequence, III, thereto (5'-TTT), as is clear from ~~figures 7(a) to 7(e)~~Figures 9A, (a) to (e).

Replace the paragraph beginning on page 31, line 14, with the following rewritten paragraph:

The sequence 5'-TTT is not however itself used to provide a sequence complementary thereto, as is clear from ~~figures 7 (f) to 7 (k)~~Figures 9A, (f) to (h) and Figure 9B,

(i) to (k). It can be seen from Figure ~~7(1)~~9B, (l) that only one of the four immobilised strands shown after two rounds of primer extension and a strand separation step comprises the additional sequence 5'-TTT and that no strand comprising a complementary sequence (5'-AAA) to this sequence is present (i.e. only one strand significantly larger than the others is present). After several rounds of amplification the strand comprising the sequence 5'-TTT will represent an insignificant proportion of the total number of extended, immobilised nucleic acid molecules present.

Replace the paragraph beginning on page 32, line 6, with the following rewritten paragraph:

~~Figure 8 illustrates~~Figures 10A and 10B illustrate one method of synthesising additional nucleic acids using immobilised nucleic acids as a starting point.

Replace the paragraph beginning on page 32, line 8, with the following rewritten paragraph:

Colonies will usually comprise both a given nucleic acid strand and its complement in immobilised form (~~figure 8a~~Figure 10A, (a)). Thus they can be used to provide additional copies not only of a given nucleic acid strand but also of its complement.

Replace the paragraph beginning on page 32, line 12, with the following rewritten paragraph:

One way of doing this is to provide one or more primers (primers TTA and TGG) in solution that anneal to amplified, immobilised nucleic acid strands present in colonies (~~figure 8c~~Figure 10A, (c)) provided by the present invention. (These primers may be the same as primers initially used to provide the immobilised colonies, apart from being provided in free rather than immobilised form.) The original DNA colony is denatured by heat to it single-stranded form (~~figure Bb~~Figure 10A, (b)), allowing primers TTA and TGG to anneal to the available 31 end of each DNA strand. Primer extension, using AmpliTaq DNA polymerase and the four deoxyribonucleoside triphosphates (~~labelled~~labeled or unlabeled) can then be used to synthesise complementary strands to immobilised nucleic acid strands or at least to parts thereof (step (iii)).

Replace the paragraph beginning on page 32, line 24, with the following rewritten paragraph:

Once newly formed strands (~~figure 8d~~Figure 10B, (d)) have been synthesised by the process described above, they can be separated from the immobilised strands to which they are hybridised (e.g. by heating). The process can then be repeated if desired using the PCR reaction, to provide large number of such strands in solution (~~figure Be~~Figure 10B, (e)).

Replace the paragraph beginning on page 33, line 24, with the following rewritten paragraph:

Oligonucleotides (Microsynth GmbH Balgach, Switzerland) were grafted onto Nucleolink wells (Nunc, Denmark). Oligonucleotide PI corresponds to the sequence 5'-TTTTTTTTTTCACCAACCCAAACCAACCCAAACC (SEQ ID NO:9) and oligonucleotide P2 corresponds to 5'TTTTTTTTTTAGAAGGAGAAGGAAAGGGAAAGGG (SEQ ID NO:10). In each Nucleolink well, a 45 µl of 10 mM 1-methyl-imidazole (pH 7.0) (Sigma Chemicals) solution containing 360 fmol of Pl and 360 fmol of P2 was added. To each well, 15 µl of 40 mM 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (pH 7.0) (Sigma Chemicals) in 10 mM 1-methyl-imidazole, was added to the solution of oligonucleotides. The wells were then sealed and incubated at 50°C for 16 hours. After the incubation, wells have been rinsed twice with 200 µl of RS (0.4N NaOH, 0.25% Tween 20), incubated 15 minutes with 200 µl RS, washed twice with 200 µl RS, and twice with 200µl TNT (100mM Tris/HCl pH7.5, 150mM NaCl, 0.1% Tween 20), before they are put to dry at 50°C in an oven. The dried tubes were stored in a sealed plastic bag at 4°C.

Replace the paragraph beginning on page 35, line 4, with the following rewritten paragraph:

PCR (25 cycles, 30 sec. at 94°C , 45 sec. at 60°C, 45 sec. at 72°C) has been performed in the Nucleolink wells with 0.25 μM primers P70 (5'-CACCAACCCAAACCAACCCAAACCACGACTCACTATAGGGCGAA (SEQ ID NO:11)) and P71 (5'-AGAAGGAGAAGGAAAGGGAAAGGGTAAAGGGAACAAAAGCTGGA (SEQ ID NO:12)) in solution in sets A. B, C and D. P70 and P71 are suited for the amplification of both S1 and S2, since primer P70 contains the sequence of primer P1 and p71 contains P2. In the set E wells, PCR has been performed with a set of forward (P150, 5'-GGTGCTGGTCCTCAGTCTGT (SEQ ID NO:13)) and reverse (P151, 5'-CCCGCTTACCAGTTTCCATT (SEQ ID NO:14)) primers which are within S1 and not within S2 so as to produce a 321 bp PCR product, and in the set F wells, PCR has been performed with a set of forward (P152, 5'-CTGGCCTTATCCCTAACAGC (SEQ ID NO:15)) and reverse (P153, 5'-CGATCTTGGCTCATCACAAT (SEQ ID NO:16)) primers which are within S2 and not within S1 so as to produce a 390 bp PCR product. For each of the 18 PCR reactions, 3 μl of solution have been used for gel electrophoresis on 1% agarose in presence of 0.1 μg/ml ethidium-bromide. The pictures of the gels are presented in ~~Figure 9~~Figures 11A and 11B. These pictures show that DNA in the colonies is protected from exonuclease digestion (sets B, C and D as compared to set A), and that both S1 and S2 can be recovered either simultaneously using P1 and P2 (sets A, B, C

and D) or specifically (set E and F). In set E and F, where the shorter PCR products are more efficiently amplified than the longer PCR products in sets A, B, C, D, a cross-contamination between the S1 and S2 templates is detectable (see lane E2 and F1).

Replace the paragraph beginning on page 36, line 10, with the following rewritten paragraph:

~~Figure 10~~Figures 12A, 12B and 12C shows how 'secondary primers' are generated using existing primary colonies. As a starting point, the primary colony (~~figure 10a~~Figure 12A, (a)) is left in the fully hybridised, double-stranded form. A single-strand specific DNA exonuclease might be used to remove all primers which have not been elongated. One could also choose to cap all free 31-OH ends of primers with dideoxyribonucleotide triphosphates using a DNA terminal transferase (step (i), ~~figure 10b~~Figure 12A, (b)).

Replace the paragraph beginning on page 36, line 18, with the following rewritten paragraph:

Secondly and independently, the DNA molecules forming the colonies can be cleaved by using endonucleases. For example, a restriction enzyme that recognises a specific site within the colony (depicted by the IRE' arrow in ~~figure 10e~~Figure 12B (c)) and cleaves the DNA colony (step (ii), ~~figure 10~~Figure 12B). If desired, the enzymatically cleaved

colony (~~figure 10d~~Figure 12B, (d)) can then be partially digested with a 3' to 51 double-strand specific exonuclease (e.g. *E. coli* exonuclease III, depicted by 'N', step (iii), ~~figure 10d~~Figure 12B, (d)). In any case, the secondary primers are available after denaturation (e.g., by heat) and washing (~~figure 10e~~Figure 12B, (e)).

Replace the paragraph beginning on page 36, line 28, with the following rewritten paragraph:

Alternatively, the double stranded DNA forming the colonies (~~figure 10f~~Figure 12C, (f)) can be digested with the double-strand specific 3'-5' exonuclease, which digest only one strand of double stranded DNA. An important case is when the exonuclease digests only a few bases of the DNA molecule before being released in solution, and when digestion can proceed when another enzyme binds to the DNA molecule (~~figure 10g~~Fgiure 12C, (g)). In this case the exonuclease digestion will proceed until there remain only single stranded molecules which, on average, are half the length of the starting material, and are without any complementary parts (which could form partial duplexes) remaining in the single stranded molecules in a colony (~~figure 10h~~Figure 12C(h)).

Replace the paragraph beginning on page 37, line 8, with the following rewritten paragraph:

In all cases, these treatments result in single-stranded fragments grafted onto a support which correspond to the sequence of the original template and that can be used for new DNA colony growing if an appropriate new template is provided for colony initiation (~~figures 10e and 10h~~Figure 12B, (e) and Figure 12C, (h)).

Replace the paragraph beginning on page 37, line 21, with the following rewritten paragraph:

~~Figure 11 a-e~~Figures 13A and 13B show how a secondary colony can be generated when an appropriate template (TP, ~~figure 11a~~Figure 13A, (a)) is provided for a second round of DNA colony generation on a support for secondary colony growing, holding secondary primers. In this example, treatment of the primary colony as described above has generated the secondary primers, SP1 and SP2 (~~figure 11a~~Figure 13A, (a)). The template TP, will hybridise to its complementary secondary primer, SP1, and following an extension reaction using a DNA polymerase as described, will be extended as depicted (~~figure 12b~~Figure 13A, (b)). Following a denaturing (step ii), reannealing (step iii) and DNA polymerase (step iv) cycle, a replica of the original primary colony will be formed (~~figure 11e~~Figure 13B, (e)).

Replace the paragraph beginning on page 39, line 2, with the following rewritten paragraph:

~~Figure 12 shows~~Figures 14A and 14B show how extended primers can be generated on an array of oligonucleotides. The same procedure could be applied to a support covered with colonies or secondary primers as described in section E.

Replace the paragraph beginning on page 39, line 5, with the following rewritten paragraph:

In ~~Figure 12a)~~Figure 14A, (a) a support is provided having a plurality of immobilised primers shown thereon. Different immobilised primers are shown present in different regions of the support (represented by squares). Primers having the sequence 5'-AAA are present in one square and primers having the sequence 5'-GGG are present in another square.

Replace the paragraph beginning on page 39, line 11, with the following rewritten paragraph:

~~Figures 12b) to 12d)~~Figure 14A, (b) and Figure 14B, (c) and (d) show how the initial primers present (initial primers) are modified to give different primers (extended primers). In this example, those initial primers having the sequence 5'-AAA are modified to produce two different types of extended primers, having the sequences 5'-AAAGCC and 5'-AAATAC respectively. This is achieved by the hybridisation of oligonucleotide templates, 5'-GTATTT and 5'-GGCTTT to the primary primers immobilised on the surface (~~figure 12b~~Figure

14A, (b)), followed by DNA polymerase reaction. Those initial primers having the sequence 5'-GGG are modified to produce two different types of extended primers, having the sequences 5'-GGGTAT and 5'-GGGTAA (~~figure 12d~~Figure 14B, (d)) in a similar manner.

Replace the paragraph beginning on page 40, line 12, with the following rewritten paragraph:

The DNA of interest is first extracted from the biological sample and cut randomly into "small" pieces (e.g., 50 to 10,000 bases long, but preferentially 500 to 1000 base pairs in length, represented by bar 'I', ~~figure 13a~~Figure 15, (a)). (This can be done e.g., by a phenol-chloroform extraction followed by ultrasound treatment, mechanical shearing, by partial digestion with frequent cutter restriction endonucleases or other methods known by those skilled in the art). In order to standardise experimental conditions, the extracted and cut DNA fragments can be size-fractionated, e.g., by agarose gel electrophoresis, sucrose gradient centrifugation or gel chromatography. Fragments obtained within a single fraction can be used in providing templates in order to reduce the variability in size of the templates.

Replace the paragraph beginning on page 40, line 25, with the following rewritten paragraph:

Secondly, the extracted, cut and (optionally) sorted template DNA fragments can be ligated with oligonucleotide linkers (IIa and IIb, ~~figure 13a~~Figure 15, (a)) containing the sequence of the primer(s) which have previously been grafted onto a support. This can be achieved, for instance, using "blunt-end" ligation. Alternatively, the template DNA fragments can be inserted into a biological vector at a site that is flanked by the sequence of the primers that are grafted on the support. This cloned DNA can be amplified within a biological host and extracted. Obviously, if one is working with a single primer grafted to the solid support for DNA colony formation, purifying fragments containing both P1 and P2 primers does not pose a problem.

Replace the paragraph beginning on page 41, line 7, with the following rewritten paragraph:

Hereafter, the DNA fragments obtained after such a suitable process are designated by the expression: "prepared genomic DNA" (III, ~~figure 13a~~Figure 15, (a)).

Replace the paragraph beginning on page 41, line 15, with the following rewritten paragraph:

The procedure is the same as that described in the previous section except that in this case, the oligonucleotide linkers used to tail the randomly cut genomic DNA fragments are now made of two parts; the sequence of the primers grafted

onto the surface (P1 and P2, ~~figure 13b~~Figure 15, (b)) and a "tag" sequence which is different for each sample and which will be used for identifying the origin of the DNA colony. Note that for each sample, the tag may not be unique, but a plurality of tags could be used. Hereafter, we will designate the DNA fragments obtained after such a suitable process by the expression "tagged genomic DNA" (III, Figure ~~13b~~15, (b)).

Replace the paragraph beginning on page 42, line 5, with the following rewritten paragraph:

The DNA of interest can first be extracted from a biological sample by any means known by those skilled in the art (as mentioned supra). Then the specific sequences of interest can be amplified with PCR (step (i), ~~figure 13e~~Figure 15 (c)) using PCR primers (IIa and IIb) made of two parts; 1) at the 5'-end, the sequences corresponding to the sequences of primer oligonucleotide(s) that have been grafted onto a surface (P1 and P2)and 2) at the 3'-end, primer sequences specific to the sequence of interest (S1 and S2). Hereafter, we will designate the DNA fragments obtained after such a suitable process by the expression: "prepared DNA" (111, ~~figure 13e~~Figure 15 (c)).

Replace the paragraph beginning on page 42, line 17, with the following rewritten paragraph:

The procedure is the same as in the previous section except that in this case the DNA primers (IIa and IM) used to perform the PCR amplification (step (i), ~~figure 13d~~Figure 13, (D)) are now made of three parts; 1) the sequence of the primers grafted onto the surface (P1 and P2), 2) a "tag" sequence which is different for each sample and which will be used for the identifying the origin of the DNA colony and 3) primer sequences surrounding the specific sequence of interest (S1 and S2). Note that for each sample, a plurality of tags might be used, as in (ii) *supra*.

Replace the paragraph beginning on page 42, line 26, with the following rewritten paragraph:

Hereafter, we will designate the DNA fragments obtained after such a suitable process by the expression: "tagged DNA" (III, ~~figure 13d~~Figure 13, (d)). Potential uses of tags are the same as in (ii), *supra*.

Replace the paragraph beginning on page 46, line 9, with the following rewritten paragraph:

In a preferred embodiment of the present invention colonies are treated with a DNA restriction endonuclease which is specific either for a sequence provided by a double stranded form of one of the primers originally grafted onto the surface where colonies are formed or for another sequence

present in a template DNA molecule (see e.g. ~~figure 16e~~Figure 12B, (c)).

Replace the paragraph beginning on page 46, line 20, with the following rewritten paragraph:

In another embodiment the colonies can be partially digested with a double-strand_specific 3' to 5' DNA exonuclease (see section E, ~~figure 10f~~Figure 12C, (f)) which removes one strand of DNA duplexes starting from the 3' end, thus leaving a part of a DNA molecule in single stranded form.

Replace the paragraph beginning on page 48, line 2, with the following rewritten paragraph:

In this embodiment, DNA colonies can be used as templates for in situ RNA synthesis as depicted in ~~figure 14~~Figure 16, (a). DNA colonies can be generated from templates and primers, such that a RNA polymerase promoter sequence is positioned at one end of the double-stranded DNA in the colony. DNA colonies can then be incubated with RNA polymerase and the newly synthesised RNA (cRNA) can be assayed as desired. The detection can be done non-specifically (e.g., staining) or in a sequence dependent way (e.g., hybridisation).

Replace the paragraph beginning on page 48, line 10, with the following rewritten paragraph:

The DNA template (I, ~~figure 14a~~Figure 16, (a)) to be amplified into A colony is generated by PCR reaction using primers Ma and IIb) which have the following four parts; 1) sequence identical to the sequences of the primers grafted onto the surface ('P1' and 'P2'), 2) a "tag" sequence which is different for each sample , a sequence corresponding to a RNA polymerase promoter, ~~ie.~~i.e., the T3, T7 and SP6 RNA promoters, ('RPP', ~~figure 14a~~Figure 16, (a)) and 4) primer sequences surrounding the-specific sequence of interest 'S1' and 'S2'). Hereafter, we will designate the DNA fragments obtained after such a suitable process by the expression: "tagged RNA synthesis DNA" (III, ~~figure 14b~~Figure 16, (b)).

Replace the paragraph beginning on page 48, line 20, with the following rewritten paragraph:

After amplification of the DNA template from the original DNA sample, these templates are used to generate DNA colonies. The DNA colonies (IV, ~~figure 14c~~Figure 16, (c)) are then incubated with the RNA polymerase specific for the RNA polymerase promoter ('RPP', Figure 16, (c)~~figure 14c~~). This will generate a copy of RNA specific for the DNA colony template (Template-cRNA, V, Figure 16, (d)~~figure 14d~~).

Replace the paragraph beginning on page 51, line 17, with the following rewritten paragraph:

If colonies are provided initially in a form comprising double-stranded molecules the colonies can be processed to provide single-stranded molecules for use in sequencing as described above. (It should however be noted that double stranded molecules can be used for sequencing without such processing. For example a double stranded DNA molecule can be provided with a promoter sequence and step-by-step sequencing can then be performed using an RNA polymerase and ~~labelled~~labeled ribonucleotides (cf ~~Figure 16 d~~Figure 16, (d))). Another alternative is for a nick to be introduced in a double stranded DNA molecule so that nick translation can be performed using ~~labelled~~labeled deoxyribonucleotides. and a DNA polymerase with 5' to 3' exonuclease activity.)

Replace the paragraph beginning on page 51, line 29, with the following rewritten paragraph:

One way of processing double-stranded molecules present in colonies to provide single-stranded colonies as described later with reference to ~~Figure 19~~Figure 18. Here double-stranded immobilised molecules present in a colony (which may be in the form of bridge-like structures) are cleaved and this is followed by a denaturing step. (Alternatively a denaturing step could be used initially and could be followed by a cleavage step). Preferably cleavage is carried out enzymatically. However other means of cleavage

are possible, such as chemical cleavage. (An appropriate cleavage site can be provided in said molecule.) ~~.~~ Denaturing can be performed by any suitable means. For example it may be performed by heating and / or by changing the ionic strength of a medium in the vicinity of the nucleic acid molecules.

Replace the paragraph beginning on page 63, line 14, with the following rewritten paragraph:

Figure ~~16~~ 18 depicts an example of *de novo* DNA sequencing. In this example, genomic DNA is fragmented into pieces of 100 to 2000 base pairs (see preparation of random DNA fragments, section G(i)). These fragments will be ligated to oligonucleotide linkers (IIa and IIb, ~~figure 16a~~Figure 18, (a)) which include sequences specific for the grafted primers on the surface ('P1' and 'P2'), a sequence which is recognised by a rare-cutting restriction nuclease ('RE') and a sequence corresponding to a sequencing primer ('SP'), resulting in templates (III, ~~figure 16b~~Figure 18, (b)). Using this prepared DNA as template for DNA colony formation, one obtains primary colonies (IV, ~~figure 16c~~Figure 18, (c)). These colonies are then digested with the corresponding restriction endonuclease and denatured to remove the non-attached DNA strand (V, ~~figure 16d~~Figure 18, (d)). The sequencing primer (SP)'is then annealed to the attached single-stranded template (~~figure 16e~~Figure 18, (e)). Incorporation and detection of

~~labelled~~labeled nucleotides can then be carried out as previously described (see section I(iii), Methods of Sequencing).

Replace the paragraph beginning on page 64, line 4, with the following rewritten paragraph:

Its preferred embodiment is depicted in ~~figure 17~~Figure 19.

Replace the paragraph beginning on page 64, line 5, with the following rewritten paragraph:

Firstly, primary colonies are prepared, as depicted in figure 3. in its preferred form, the DNA used for this preparation is 'prepared genomic DNA' or 'tagged genomic DNA', as described in section G(i) and G(iii), respectively, and where the DNA is either from the whole genome of one (or several) organism(s) or from a subset thereof (e.g., from a library of previously isolated genes). In ~~figure 17~~Figure 19, the uppercase letters, "A", "B" and "D" represent colonies which have arisen from genes which exhibit high, medium and low expression levels, respectively, and "E" represents colonies arising from non-expressed genes (in real cases, all these situations may not necessarily be present simultaneously).

Replace the paragraph beginning on page 64, line 16, with the following rewritten paragraph:

Secondly, the colonies are treated to turn then into supports (i.e. secondary primers) for secondary colony growing (step i in ~~figure 17a~~Figure 19, (a)), as described in section E. At this stage (Figure 19, (a)~~figure 17b~~), the treated colonies are represented by underlined characters (A, B, D, or E).

Replace the paragraph beginning on page 64, line 21, with the following rewritten paragraph:

Thirdly, (step ii in ~~figure 17b~~Figure 19, (b)) this support for secondary colony growing is used to regenerate colonies from mRNA (or cDNA) templates extracted from a biological sample, as described in section C. If the template is mRNA, the priming step of colony regeneration will be performed with a reverse transcriptase. After a given number of colony amplification cycles, preferably 1 to 50, the situation will be as depicted in (Figure 19, (c)~~figure 17c~~): the colonies corresponding to highly expressed genes (represented by the letter "A") are totally regenerated, as their regeneration has been initiated by many copies of the mRNA; the colonies corresponding to genes of medium expression levels (represented by the letters "b" and "B"), have been only partially regenerated; only a few of the colonies corresponding to rare genes (represented by the letter "d"), have been partially regenerated; the colonies corresponding to

non-expressed sequences (represented by the letter "E"), have not been regenerated at all.

Replace the paragraph beginning on page 65, line 8, with the following rewritten paragraph:

Lastly, (step iii in ~~figure 17c~~Figure 19, (c)), additional cycles of colony growing are performed (preferably 2 to 50), and the colonies which have not been totally regenerated during the previous steps finally become totally regenerated, "b" becomes "B", "d" becomes "D" (Figure 19, (d) ~~figure 17d~~) the colonies corresponding to genes with high and medium expression levels are all regenerated "A" and "B" or "B"; the colonies corresponding to genes with low levels of expression are not all regenerated "D" and "D"; the colonies corresponding to non-expressed sequences are not regenerated at all "E".

Replace the paragraph beginning on page 67, line 11, with the following rewritten paragraph:

The preferred embodiment for isolating genes from a specific or activated biological sample (hereafter called target sample) which are up-regulated compared with a reference biological ~sample (hereafter called reference sample) is depicted in ~~figure 18~~Figure 20.

Replace the paragraph beginning on page 18, line 15, with the following rewritten paragraph:

Firstly, primary colonies are prepared (~~figure 18a~~Figure 20, (a)). In its preferred form, the DNA used for this preparation is prepared genomic DNA or tagged genomic DNA, as described in sections G(i) and G(ii), respectively, where the DNA is either from the whole genome of one (or several) organism(s) or from a subset thereof (e.g., from a library of previously isolated genes), and where both the primers used for colony generation (hereafter called P1 and P2) contain a endonuclease restriction site. In Figure 20, (a) ~~figure 18a~~, "A" represents colonies which have arisen from genes expressed in both the reference sample and the target sample, "B" represents colonies which have arisen from genes expressed only in the reference sample, "C" represents colonies which have arisen from genes expressed only in the target sample, and "D" represents colonies arising from non-expressed genes (in real cases, all these situations may not necessarily be present simultaneously).

Replace the paragraph beginning on page 68, line 1, with the following rewritten paragraph:

Secondly, primary colonies are then treated to generate secondary primers as the support for secondary colony growing (step i in ~~figure 18a~~Figure 20, (a)). At this stage (b), the colonies are represented as underlined characters (A,B,C,D).

Replace the paragraph beginning on page 68, line 5, with the following rewritten paragraph:

Thirdly, (step ii in ~~figure 21b~~Figure 20, (b)) the secondary primers are used to regenerate colonies using mRNA or cDNA (represented by "mA + mB") extracted from the biological reference sample as a template, as described in G(v). If the template is mRNA, the first elongation step of colony regeneration will be performed with a reverse transcriptase. After enough colony growing cycles, preferably 5 to 100, only the colonies corresponding to genes expressed in the reference sample ("A" and "B") will be regenerated, as depicted in (Figure 20, (c)~~figure 18c~~).

Replace the paragraph beginning on page 68, line 14, with the following rewritten paragraph:

In step (iii), the colonies are digested with a restriction enzyme (represented by RE) which recognises a site in the flanking primer sequences, P1 and P2, which are grafted on the support and which were the basis of primary colony generation. Importantly, only the colonies which have been regenerated during step (ii) will be digested. This is because the support for secondary colony growth is made of single stranded DNA molecules, which can not be digested by the restriction enzyme. Only the regenerated colonies are present in a double stranded form, and are digested. After

digestion, the situation is the one depicted in ~~figure 18d~~Figure 20, (d). The colonies corresponding to the genes expressed in the reference sample have totally disappeared, i.e., they are not even present as a support for secondary colony growth, and the colonies corresponding to genes expressed only in the target sample "C" and the colonies corresponding to non-expressed genes "D" are still present as a support for secondary colony generation.

Replace the paragraph beginning on page 68, line 30, with the following rewritten paragraph:

In step (iv), mRNA (or cDNA) (represented by "mA + mC") extracted from the target sample is used to generate secondary colonies. Because colonies corresponding to mA and mB no longer exist, only the colonies corresponding to mC can be regenerated (~~ie. Only~~i.e., only the mRNA specifically expressed in the target sample). After sufficient number of colony growing cycles (preferably 5 to 100), the situation is such that only the colonies corresponding to genes expressed specifically in the target sample are regenerated ("C", ~~figure 18e~~Figure 20, (e)).

Replace the paragraph beginning on page 69, line 12, with the following rewritten paragraph:

The preferred embodiment for isolating genes from a specific or activated biological sample which are less

expressed than in a reference biological sample is depicted in ~~figure 19~~Figure 21. The different steps involved in this procedure are very similar to those involved in the isolation of gene which are more regulated than in the reference sample, and the notation are the same as in ~~figure 18~~Figure 20. The only difference is to inverse the order used to regenerate the colonies: in step (ii), the mRNA used is the one extracted from the target biological sample ("mA + mC") instead of the mRNA extracted form the reference biological sample ("mA + mB"), and in step (iv), the mRNA used is the one extracted from the reference biological sample ("mA + mB") instead of the one extracted from the target sample ("mA + mC"). As a result, only the DNA from colonies corresponding to genes which are expressed in the reference sample but not in the target sample is recovered and amplified ("B", ~~figure 19f~~Figure 21, (f)).